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Investigation of zinc-containing peptide deformylase from *Leptospira interrogans* by X-ray absorption near-edge spectroscopy

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Peptide deformylase (PDF, EC 3.5.1.27) is essential for the normal growth of eubacterium but not for mammalians. Recently, PDF has been studied as a target for new antibiotics. Its activity is strongly dependent on the bound metal ion. The crystallographic studies did not show any significant structural difference upon various bound metal ions. In this paper, X-ray absorption spectroscopy was employed to determine the local structure around the zinc ion of PDF from *Leptospira interrogans* in dry powder. XANES (X-ray absorption near-edge structure) calculations were performed and the local geometry of the active center was reconstructed successfully. By comparing with the crystal structure of an enzyme-product complex, the results from calculations show that a water molecule has moved towards the zinc ion and lies in the distance range to coordinate with the zinc ion weakly.

Keywords: peptide deformylase; metalloproteinases; catalytic mechanism; BioXAS.

1. Introduction

Peptide deformylase (PDF; EC 3.5.1.27), as a new member of the zinc metalloproteases superfamily, is an enzyme responsible for the removal of a formyl group from nascent polypeptides (Adams, 1968; Livingston & Leder, 1969). This deformylation process is unique for bacteria and does not exist in mammalian protein synthesis, so PDFs have been extensively studied as a new target of structure–mechanism-based antibiotics. Therefore, an improved understanding of the catalytic mechanism is the pre-requisite for the development of new drugs.

PDF utilizes a metal ion to effect the hydrolysis of an amide bond. In the ground state of the native enzyme structure, the metal ion is tetrahedrally coordinated by two histidines from a conserved HEXXH motif, a cysteine and a water molecule. Several kinds of metal ions including zinc (Meinnel & Blanquet, 1995; Durand *et al.*, 1999), cobalt (Durand *et al.*, 1999), nickel (Dardel *et al.*, 1998; Ragusa *et al.*, 1998) and iron (Rajagopalan *et al.*, 1997) have been found in preparations of purified PDFs. Moreover, the specific activity of PDF greatly depends on the metal ion located in its active site. For example, Zn-containing *E. coli* PDF is much less active than iron-, nickel- or cobalt-containing PDFs. However, peptide deformylase from a real pathogenic bacterium, *Leptospira interrogans* (*Li*PDF), is the first bacterial zinc PDF with high activity. When the zinc ion was replaced by an iron, cobalt or nickel ion, the activity decreased. Up to now, although several crystal (X-ray) or NMR structures of PDFs have been determined, no direct structural explanations are available for such enormous metal-dependent activity differences. The major reason is that, at current crystallographic resolution, the three-dimensional structures of different metal-binding PDFs seem identical.

X-ray absorption spectroscopy (XAS) is a unique tool for determining the local structure of metal-containing proteins at ultrahigh resolution and can provide subtle structural variation information precisely (Hasnain & Hodgson, 1999; Hasnain & Strange, 2003). Here we report a preliminary study using the XAS technique to detect the fine structure of the *Li*PDF active center, expecting an improved understanding of the catalytic mechanism.

2. Experiment

*Li*PDF has been over expressed in *E. coli* and purified according to the protocol previously reported (Zhou & Gong, 2004). Expression was performed in 1 l LB medium (100 μ*M* $ZnCl_2$ added) and incubated at 310 K. When OD600 reached 0.6, the cultures were induced by the addition of a final concentration of 800 μ*M* IPTG at 310 K for an additional 4 h. The cells from 1 l of culture were harvested in 20 ml of cold (277 K) buffer A (50 m*M* HEPES pH 7.5, 10 m*M* NaCl, 100 μg ml^{-1} phenylmethanesulfonyl fluoride, 100 μ*M* $ZnCl_2$) and then lysed by sonication while keeping on ice. The clear supernatant was collected by centrifugation and applied to a DEAE-sepharose column equilibrated with 200 ml buffer B (50 m*M* tris-HCl pH 8.0, 10 m*M* NaCl, 100 μ*M* $ZnCl_2$). The column was eluted with 250 ml of buffer B plus a linear gradient of 10–500 m*M* NaCl. The fractions with PDF activity were concentrated and applied to a Superdex G-75 column pre-equilibrated with buffer C (50 m*M* tris-HCl pH 8.0, 50 m*M* NaCl, 100 μ*M* $ZnCl_2$). For XAS studies, the purified protein was dialyzed against pure water and then was lyophilized to dry powder. No formate salts were added during the expression and purification process.

X-ray absorption spectra at the Zn *K* edge were collected in 'fluorescence yield' (FY) mode at the X-ray absorption station (beamline 1W1B) of the Beijing Synchrotron Radiation Facility (BSRF). The storage ring was operating at the typical energy of 2.2 GeV with the current decreasing approximately from 135 mA to 80 mA during a time span of 8 h. To suppress unwanted harmonics, a detuning of 30% was performed between the two crystals of the monochromator. The incident-beam intensity was monitored and recorded using an ionization chamber filled with a 25% argon-doped nitrogen mixture, and the fluorescence signal was collected and recorded using a fluorescence ionization chamber filled with argon gas.

3. Results and discussion

The theoretical *ab initio* calculation of the XANES region is of great interest for structural studies of biological systems. In fact, despite the fact that the magnitude of the scattering amplitudes from light elements (which biological systems are mostly made of) severely limits the energy range of the available experimental data, the precise form of the spectrum in the low-energy region is extremely sensitive to a variety of structural details that are often crucial for our understanding of the subtle relations between structure and function. In this context, a wealth of information has been obtained by several XAS studies on active sites in metalloproteins (Hasnain & Hodgson, 1999; Mijovilovich & Meyer-Klaucke, 2003).

In order to extract detailed structure information around the zinc ion site, a new approach implemented by a code called *MXAN* (*MINUIT* XANes) was introduced (Della Longa *et al.*, 2001; Benfatto & Wu, 2003; Cardelli *et al.*, 2003), which is capable of yielding a quantitative analysis of the spectrum from the absorption edge up to 200 eV through fitting the reconstructed plot from *ab initio* calculation to the experiment data by adjusting the input structural model. This approach has the power of distinguishing the contributions between water molecular and oxygen atoms (Benfatto *et al.*, 1997, 2004; D'Angelo *et al.*, 2002). This method takes into account multiple-scattering (MS) events in a rigorous way through the evaluation of the scattering path operator (Natoli & Benfatto, 1986; Wu *et al.*, 1996), and its effectiveness has been successfully tested in a number of interesting situations (Della Longa *et al.*, 2001; Benfatto *et al.*, 2003).

Fig. 1 shows the experimental Zn *K*-edge X-ray absorption near-edge spectrum (solid line) of a dry powder sample of *Li*PDF. We denote the well resolved peak features as A, B, C and D. The peaks A and B are mainly due to the MS resonances of the excited photoelectrons, scattered by the first neighbor atoms. Besides the experimental data, the reconstructed plot is also given in Fig. 1 (circles), which has been computed directly from the structure model. As there is no suitable crystallographic structure of *Li*PDF available until now, the initial structure model was based on the crystal structure of *E. coli* zinc-containing PDF (PDB entry code: 1BS5) because of the similarity of local geometry in the active center. As we can see, the MS calculation reproduces all the main features of the experimental spectrum, indicating that the atomic cluster, containing 43 atoms, is large enough to characterize qualitatively the spectral features. However, the discrepancy between the experimental data and the MS calculation reflects that the environment around the zinc ion should be different from the initial structure.

The best-fitting result was reached by moving all the first-shell atoms of the initial structure model, and no linkage was set between those atoms. Fig. 2 presents a comparison of the fitted curve with the experimental data. The square residue values for calculations shown in Figs. 1 and 2 were 10.80 and 3.90, respectively. Some refined critical distances are listed in Table 1. For comparison, those data of *E. coli* PDF as the initial model, as well as those of the catalysis product of *Li*PDF, are also listed in Table 1. According to the results of the calculation, the structure of the active center of *Li*PDF in its native state has been plotted in Fig. 3(*a*).

The crystal structure of *Li*PDF has already been reported (Zhou & Gong, 2004). However, this crystal was obtained with 4 *M* formate; therefore it is the product of *Li*PDF catalysis, in which a formate group binds with the zinc ion (PDB entry code: 1RN5; see Fig. 3*b*). Since the sample used for XAS was dialyzed against pure water, the metal center structure of *Li*PDF determined by XAS is of the native state, in which a water molecule Wat0 coordinates to the zinc ion, instead of a formate (see Fig. 3*a* in detail). Because it is very difficult to obtain the crystallized sample of *Li*PDF, the structure of the active center of *Li*PDF in its native state has been resolved by XAS methods, and the XANES fitting results also confirmed the conservation of the metal core structure with standard coordinating distances. Unexpectedly, another conserved water molecule Wat1 (from a conserved three-molecule water chain near the zinc ion), that stabilizes the active center in the crystal structure of *Li*PDF-formate (Fig. 3*b*), was observed to

Table 1
Some critical distances in three structures.

The first group shows the theoretical results calculated using *MXAN*. For comparison, the catalysis product of *Li*PDF (formate coordinated) and the *E. coli* PDF of the native state (the initial structure model used for calculation) are also listed, in the second and third groups.

Structure	Atom pair		Distance (Å)
*Li*PDF XAS structure (native state)	Zinc	Cys101-SG	2.21
	Zinc	His143-NE2	2.08
	Zinc	His147-NE2	2.08
	Zinc	Wat0-OH2	2.24
	Zinc	Wat1-OH2	2.78
*Li*PDF crystal structure (formate coordinated, PDB entry code 1RN5)	Zinc	Cys101-SG	2.40
	Zinc	His143-NE2	2.13
	Zinc	His147-NE2	2.15
	Zinc	Formate-O1	2.58
	Zinc	Formate-O2	2.31
	Zinc	Wat1-OH2	3.48
E. coli PDF crystal structure (native state, PDB entry code 1BS5)	Zinc	Cys90-SG	2.13
	Zinc	His132-NE2	2.02
	Zinc	His136-NE2	2.16
	Zinc	Wat0-OH1	2.25
	Zinc	Wat1-OH2	3.14

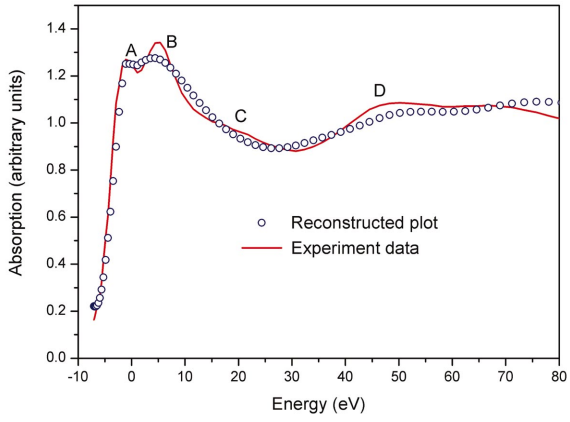

Figure 1
Experimental data and the theoretically reconstructed plot of the Zn *K*-edge X-ray absorption near-edge spectrum of *Li*PDF in dry powder state. The theory spectrum was computed directly from the initial structure model.

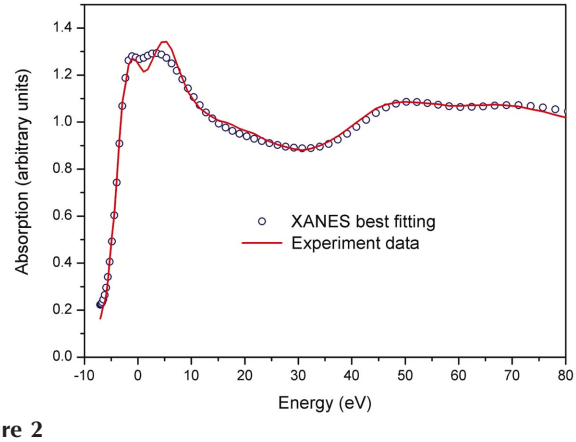

Figure 2
Comparison between the best-fit result and the experimental data for a dry powder sample of *Li*PDF. The theoretical result is obtained by moving all the first-shell atoms independently.

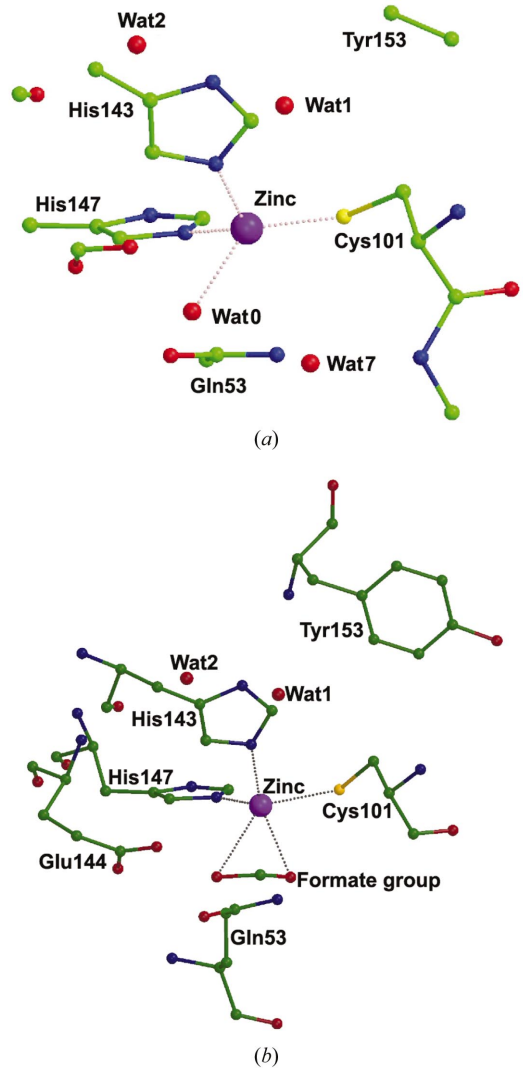

Figure 3
Local structure of the active center in *Li*PDF. (*a*) The active center calculated from *Li*PDF XANES fitting with carbon in green, nitrogen in blue, oxygen in red and sulfur in yellow. The coordination of the zinc ion (in purple) is indicated by the pink dashed line. A water molecule (Wat0) coordinates with the zinc ion, and another water molecule (Wat1) lies at a distance within the range to coordinate with the metal ion weakly. (*b*) The active center of the formate-coordinated *Li*PDF with carbon in dark green, nitrogen in dark blue, oxygen in dark red and sulfur in dark yellow. The purple sphere corresponds to the zinc ion and its coordination is indicated by the black dashed line. A formate group was observed to coordinate with the zinc ion.

move towards the zinc ion in this structure, so that it lies in the distance range to coordinate with the metal ion weakly.

4. Conclusion

We have shown, for the first time, that the active center structure of *Li*PDF can be defined quantitatively by a new analysis procedure of *MXAN*. Full MS analysis of the Zn *K*-edge XANES spectrum, taking into account a cluster including 43 atoms lying up to 6 Å from the central zinc atom, can reproduce all features in the near-edge region and give detailed structural information around the zinc site. Through

comparison of *ab initio* full MS computations and experimental data at the Zn *K* edges, we have found that the water molecule Wat1 moves towards the zinc ion in this structure, lying in the distance range to coordinate with the metal ion weakly. If this movement is necessary for the activity of PDF, there is a need for further studies on *Li*PDF containing different metal ions and/or in solution state. This work is in progress and will be addressed in a forthcoming paper.

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